Protocol for a PCR reaction using NEBNext® Q5® Hot Start HiFi PCR Master Mix (M0543)

Overview

Please note that protocols with NEBNext Q5 Hot Start HiFi PCR Master Mix may differ from protocols with other polymerases. The NEBNext Q5 Hot Start HiFi PCR Master Mix is the original hot start formulation of Q5 High-Fidelity DNA Polymerase that was specifically optimized for robust, high-fidelity amplification of next-generation sequencing (NGS) libraries, regardless of GC content. This has now been superseded by the NEBNext Ultra II Q5® Master Mix hot start formulation (NEB #M0544), which provides superior GC coverage.

For detailed product information visit www.neb.com/M0543

Reaction setup:
- Mix individual components prior to use
- Reactions can be assembled at room temperature

Volume per 50 ul RXN for use with NEBNext Kit Part Numbers		
Component	**E7370, E6000, E6040**	**E7420, E7530, E6100, E6110, E6200, E6240**
NEBNext® Q5® Hot Start HiFi PCR Master Mix	25 µl	25 µl
10 µM Forward Primer	5 µl*	2.5 µl*
10 µM Reverse Primer	5 µl*	2.5 µl*
NEBNext Adaptor-ligated DNA	15 µl	20 µl

* NEBNext Index Primers are supplied in E7350, E7335, E7500, E7710, E7730, E7600, E7535 (10 µM)
* For NEBNext Primers as supplied in E6609 (10 µM) use 10 µl/ 5 µl of the combined primer

Gently mix the reaction and collect the liquid at the bottom of the tube with a quick spin
Transfer PCR tubes to a preheated PCR machine and begin thermocycling

Recommended Thermocycling Conditions for a NEBNext Library Prep PCR:

NGS PCR			
Step	**Temp**	**Time**	**Cycles**
Initial Denaturation	98°C	30 seconds	1
Denaturation Annealing/Extension**	98°C 65°C	10 seconds 75 seconds	3-15*
Final Extension	65°C	5 minutes	1
Hold	4-10°C	∞	

* The number of cycles depends on the input amount and NEBNext Library Prep kit used. Refer to the table below for general guidelines. Further optimization to avoid over amplification may be required.
** For NGS primers other than NEBNext index primers the Tmn may be different. In that case use 30 seconds at Tm (use NEB Tm calculator) and 45 seconds at 65°C. Annealing temperature may need to be further optimized.

General Guidelines:

1. Use of high quality, purified DNA templates greatly enhances the success of PCR reactions. Recommended amounts of DNA template for a 50 µl reaction are as follows:

Starting Material	Product #	Input Amount per Library	Cycles
DNA	E6040	5 µg	3
		1 µg	4
	E7370	1 µg	4
		50 ng	7–8
		5 ng	12
Total RNA	E7420 and E7530	100 ng	15
		1 µg	12
ChIP DNA	E6420	10 ng	15

2. Mg++ and additives:

   The NEBNext Q5 Hot Start HiFi PCR Master Mix contains 2.0 mM Mg ++ when used at a 1X concentration. This is optimal for most PCR products generated with this master mix.

3. Deoxynucleotides:

   The final concentration of dNTPs is optimized for robust library amplification. Q5 High-Fidelity DNA Polymerase cannot incorporate dUTP and is not recommended for use with uracil containing primers or templates.

4. DNA polymerase concentration:

   The concentration of DNA Polymerase in the NEBNext Q5 Hot Start HiFi PCR Master Mix has been optimized for best results under a wide range of conditions.

5. Denaturation:

   An initial denaturation of 30 seconds at 98°C is sufficient for most sample types. During thermocycling, the denaturation step should be kept to a minimum. Typically, a 10 second denaturation at 98°C is recommended for most templates.

6. Annealing:

   For NGS primers other than NEBNext index primers the Tm may be different. In that case use 30 seconds at Tm (use

NEB Tm calculator) and 45 seconds at 65°C.

7. Extension:

   The recommended extension temperature is 65°C. Extension times are generally 30 seconds for libraries up to 1 kb. Larger insert lengths may require additional time. A final extension of 5 minutes at 65°C is recommended.

8. The numbers of cycles depends on the input amount and NEBNext Library Prep kit used. Check the kit manual for a guideline for PCR cycle numbers. Further optimization to avoid over amplification may be required.

9. PCR product:

   The PCR products generated using NEBNext Q5 Hot Start HiFi PCR Master Mix have blunt ends.

10. Bead Compatibility:

    The NEBNext Q5 Hot Start HiFi PCR Master Mix is compatible with a variety of carboxylated, tosylated and strptavidin beads that may be carried over or included in the PCR step of library construction protocols including Agencourt® AMPure® XP (Beckman Coulter, Inc.), Sera-Mag SpeedBeads and Mag-Bind® RXNPure Plus (Omega Bio-tek, Inc.). SPRI Beads or PCR purification columns are recommended for post PCR clean up.